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龙舌兰发酵叶汁中的一个新甾体皂苷*

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摘要：从龙舌兰（*Agave americana*）的发酵叶汁中分离到一个新甾体皂苷。结合波谱和化学方法，新化合物的结构鉴定为替告皂苷元 3 – O – α – L – 鼠李吡喃糖基 – (1→3) – β – D – 葡萄吡喃糖基 – (1→2) – [β – D – 葡萄吡喃糖基 – (1→3)] – β – D – 葡萄吡喃糖基 – (1→4) – β – D – 半乳吡喃糖苷。

关键词：龙舌兰；甾体皂苷
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A New Steroidal Saponin from Fermented Leaves of *Agave americana* *

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Abstract：A new steroidal saponin and two known steroidal compounds were isolated from fermented leaves of *Agave americana*. The structure of the new steroidal saponin was elucidated as tigogenin 3-O-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside by spectroscopic data and chemical method.

Key words：*Agave americana*，Steroidal saponins

Agave americana L.，is native to Mexico and widely cultivated in the south of China. Its sap is applicable as a laxative and diuretic according to the Chinese herbal description. Its leaf is also used to produce steroidal sapogenins such as hecogenin（Jiangsu New Medical College，1977）. Some steroidal saponins have been isolated from the leaves of *A. americana*（Kintya *et al*，1975；Wilkomirski *et al*，1975；Yokosuka *et al*，2000）. Several steroidal saponins from this species exhibited moderate cytotoxic activity on HL-60（Yokosuka *et al*，2000）. To investigate steroidal constituents of fermented leaves of *Agave americana* L. and discover the bioactivity compounds，we have phytochemically investigated the fermented leaves of *A. americana*. A new tigogenin glycoside together with two known steroidal compounds were isolated from the fermented leaves of *A. americana*. The known compounds **1** and **2**

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were identified as (25*R*)-5α-spirostan-3β-ol (tigogenin) (**1**) (Agrawal *et al*, 1985) and tigogeninn 3-O-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**2**) (Mimaki *et al*, 1995), respectively.

Compound **3** was obtained as an amorphous powder. Its molecular formula was determined as $C_{57}H_{94}O_{27}$ on the basis of the ^{13}C DEPT NMR spectrum and negative ion FABMS. The negative ion FAB mass spectrum of **3** exhibited a molecular ion peak at *m/z* 1109 $[M-H]^-$, and three fragment ion peaks at *m/z* 1047 $[M-H-162]^-$, 901 $[M-H-162-146]^-$ and $[M-H-162-146-162]^-$. Its ^1H spectrum showed signals for two tertiary methyl groups at δ_H 0.72 and 0.89, three secondary methyl groups at δ_H 0.76, 1.20 and 1.70. In addition, five anomeric proton signals were observed at δ_H 4.95 (1H, d, J = 7.6 Hz), 5.21 (1H, d, J = 7.9 Hz), 5.27 (1H, d, J = 7.6 Hz), 5.57 (1H, d, J = 7.9 Hz) and 6.22 (1H, br s). Broad singlet peak of δ_H 6.01 indicated the α-orientation at the anomeric center of L-rhamnose. The J values (> 7 Hz) of the other four anomers of the sugar moieties indicated the β-orientation at the anomeric center of the D-pyranoses. These ^1H NMR spectral features and a diagnostic acetal carbon signal at δ_C 109.4 were indicative of **3** being a spirostanol saponin with a sugar chain made up of five monosaccharides.

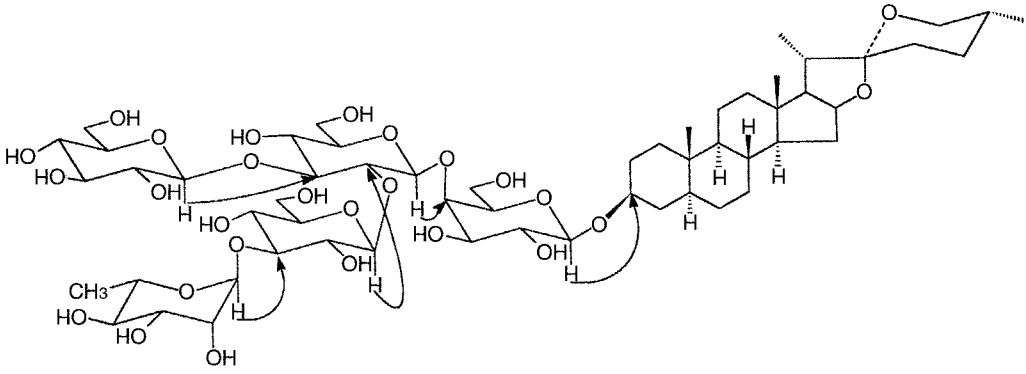

Fig. 1 The key ^1H-^{13}C long-range correlations of **3** in the HMBC spectrum

Acid hydrolysis of **3** gave tigogenin as the aglycone and the sugar residues which were identified as D-glucose, D-galactose and L-rhamnose by GC/MS.

The determination of the sequences of the sugar chain was carried out by the following NMR experiments. The easily distinguished anomeric proton signals served as the starting point analysis, the ^1H and ^{13}C NMR assignments of sugar groups of **3** were carried out by analysis of the HMQC-TOCSY and $^1H-^1H$ COSY data followed by HMQC spectrum. Three-bond ^1H-^{13}C long-range correlations in HMBC spectrum exhibited that the anomeric proton signals correlated with the carbon signals at δ_H 4.95 (H-Gal-1) and δ_C 77.6 (C-3 of aglycone), 5.21 (H-Glc-1) and 80.1 (C-Gal-4), 5.57 (H-Glc′-1) and 81.4 (C-Glc-2), 5.27 (H-Glc″-1) and 88.8 (C-Glc-3), 6.22 (H-Rha-1) and 83.3 (C-Glc′-3) (Fig. 1). Therefore, the structure of **3** was elucidated as tigogenin 3-O-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, which was named agamenoside G.

Experimental Section

Optical rotations were measured with HORIBA SEPA-300 high-sensitive polarimeter. IR (KBr) spectra were measured on Bio-Rad FTS-135 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 or Brucker AM-400 instrument at 25℃, using TMS as an internal standard. The negative ion and high-resolution FAB mass spectra were recorded on a VG AutoSpec-3000 mass spectrometer using glycerol as matrix. GC was run on Fisons MD800 GC/MS. RP-8 (Merck) was used for column chromatography; D-glucose (Merck), D-galactose (Merck), and L-rhamnose (Merck) were used as standard reagents for GC. Precoated silica gel plates (Qingdao Haiyang Chemical Co.) were used for TLC. Detection was done by spraying the plates with 5% anisaldehyde-sulphric acid, followed by heating.

The dried residues of fermented leaves of *A. americana* L. were collected from a factory in Ruili County of Yunnan Province at January 2000. The leaf-juices of *A. americana* were subjected to natural fermentative process for one year.

The dried residues of fermented leaves of *A. americana* L. (6.5 kg) were extracted with hot methanol (10 L) three times for 4 hours, the combined methanol extract was concentrated under reduced pressure. Then concentrated extract was partitioned between *n*-butanol and water. The *n*-butanol layer was repeatedly chromatographed on silica gel with $CHCl_3$: MeOH : H_2O and RP-8 with MeOH : H_2O to afford **1** (36 mg), **2** (200 mg) and **3** (378 mg).

3 (45 mg) was refluxed with 1 mol/L HCl-dioxane (1 : 1, v/v, 4 ml) on water bath for 6 h. The reaction mixture was evaporated to dryness. The dry reaction mixture was extracted by $CHCl_3$ four times. The $CHCl_3$ extract was concentrated and chromatographed on silica gel to give **3a** (11 mg), which was identified to be tigogenin with TLC by comparison with authentic sample. The sugar residues were trimethylsilylated for GC analysis.

The dry sugar residue was diluted in 5 ml pyridine without water and treated with 0.5 ml trimethyl-chlorsilan (TMCS, Fluka) at room temperature for 30 min. The reaction mixture was evaporated to dryness under reduced pressure. The mixture of trimethylsilylated derivatives of the monosaccharides was diluted in 0.5 ml MeOMe without water, which was then analyzed by GC. GC/MS: AC-5 capillary column (30 m × Φ 0.25 mm); detector: MS (270℃); column temperature: 180 ~ 260℃, rate 5℃/min; column head pressure: 12 P; carrier gas: He (distributary ratio: 30 : 1). R_t (second): D-glucose (695), D-galactose (657), and L-rhamnose (434) [authentic sample, D-glucose (685), D-galactose (650), and L-rhamnose (430)].

Tigogenin (1): A white crystal; $[\alpha]_D^{18.0}$ − 17.2° (*c* 0.036, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3519 3407, 2921, 1455, 1176, 1051, 979, 920, 900, 864 (intensity: 900 > 920); EI-MS *m/z*: 416, 344, 302, 287, 273, 139 (base peak), 122, 107, 97, 81, 67; ^{1}H NMR ($CHCl_3$-d_5, 400 MHz): δ 3.42 (1H, H − 3), 4.32 (1H, H − 16), 0.75 (3H, s, H − 18), 0.69 (3H, s, H − 19), 0.88 (3H, d, J = 7.3 Hz, H − 21), 3.47 (dd, 3.7 Hz, 11.4 Hz), 3.39 (t, 11.4 Hz), (1H each, H − 26), 0.75 (3H, d, J = 6.3 Hz, H − 27); ^{13}C NMR data see table 1.

Compound (2): A white amorphous powder; $[\alpha]_D^{26.0}$ − 48.0° (*c* 0.010, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3428, 2933, 1377, 1157, 1072, 980, 921, 898, 865 (intensity: 898 > 921); Negative ion FAB-MS *m/z* 1179 [M-H]$^-$, 1047 [M-H-132]$^-$, 871 [M-H-146-162]$^-$, 739 [M-H-132-146-162]$^-$, 577 [M-H-132-146-162-162]$^-$; ^{1}H NMR (pyridine-d_5, 400 MHz): δ 3.93 (1H, H − 3), 4.46 (1H, H − 16), 0.81 (3H, s, H − 18), 0.65 (3H, s, H − 19), 1.02 (3H, d, J = 7.4 Hz, H − 21); 3.59 (dd, 3.5 Hz, 11.0 Hz, H − 26), 3.48 (t, 11.0 Hz, H − 26), 0.68 (3H, d, J = 5.1 Hz, H − 27), 4.86 (1H, d, J = 7.4 Hz H-Gal-1), 5.15 (1H, d, J = 7.8 Hz, H-Glc-1), 5.47 (1H, d, J = 7.9 Hz, H-Glc′-1), 5.11 (1H, d, J = 7.4 Hz, H-Xyl-1), 6.09 (1H, br s, H-Rha-1), 1.61 (3H, d, J = 7.0 Hz, H-Rha-6); ^{13}C NMR data see table 1.

Compound (3): A white amorphous powder: $[\alpha]_D^{14.2}$ − 70.0° (*c* 0.0273, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3426, 2932, 2866, 1705, 1456, 1374, 1158, 1072, 982, 920, 899, 867 (intensity: 899 > 920); HR FAB-MS *m/z* 1209. 5941 [M-H]$^-$ (calcd for $C_{57}H_{93}O27$, 1209.5904); Negative ion FAB-MS *m/z* 1109 [M-H]$^-$, 1047 [M-H-162]$^-$, 901

Table 1 ^{13}C NMR data of compounds 1-3

Aglycone	1	2	3	Sugar	2	3
1	36.9 (t)	37.3 (t)	37.3 (t)	Gal 1	102.6 (d)	102.5 (d)
2	31.2 (t)	30.0 (t)	30.0 (t)	2	73.2 (d)	73.2 (d)
3	71.0 (d)	77.6 (d)	77.6 (d)	3	75.3 (d)	75.6 (d)
4	37.8 (t)	35.0 (t)	34.9 (t)	4	79.6 (d)	80.1 (d)
5	44.7 (d)	44.9 (d)	44.7 (d)	5	75.4 (d)	75.6 (d)
6	28.3 (t)	29.0 (t)	29.0 (t)	6	60.8 (t)	60.8 (t)
7	32.1 (t)	32.5 (t)	32.5 (t)	Glc 1	104.7 (d)	104.7 (d)
8	35.0 (d)	35.4 (d)	35.3 (d)	2	81.0 (d)	81.2 (d)
9	54.3 (d)	54.6 (d)	54.5 (d)	3	87.4 (d)	88.8 (d)
10	35.5 (s)	35.9 (s)	35.9 (s)	4	70.4 (d)	70.8 (d)
11	20.9 (t)	21.4 (t)	21.4 (t)	5	77.6 (d)	77.6 (d)
12	39.9 (t)	40.3 (t)	40.2 (t)	6	63.0 (t)	63.1 (t)
13	40.5 (s)	40.9 (s)	40.9 (s)	Glc′1	104.4 (d)	104.5 (d)
14	56.2 (d)	56.6 (d)	56.5 (d)	2	76.3 (d)	76.5 (d)
15	31.6 (t)	32.2 (t)	32.2 (t)	3	83.8 (d)	83.3 (d)
16	80.8 (d)	81.2 (d)	81.2 (d)	4	69.6 (d)	69.2 (d)
17	62.0 (d)	63.0 (d)	63.1 (d)	5	78.5 (d)	78.5 (d)
18	16.3 (q)	16.7 (q)	16.7 (q)	6	62.4 (t)	62.2 (t)
19	12.2 (q)	12.4 (q)	12.4 (q)	Rha 1	102.8 (d)	102.8 (d)
20	41.5 (d)	42.0 (d)	42.1 (d)	2	72.3 (d)	72.4 (d)
21	14.3 (q)	15.0 (q)	15.0 (q)	3	72.6 (d)	72.6 (d)
22	109.2 (s)	109.3 (s)	109.4 (s)	4	74.2 (d)	74.2 (d)
23	31.2 (t)	31.9 (t)	31.9 (t)	5	69.8 (d)	69.8 (d)
24	28.4 (t)	29.4 (t)	29.3 (t)	6	18.6 (q)	18.7 (q)
25	30.1 (d)	30.7 (d)	30.7 (d)	Xyl or Glc″1	104.9 (d)	104.5 (d)
26	66.7 (t)	67.0 (t)	67.0 (t)	2	75.6 (d)	75.4 (d)
27	17.0 (q)	17.4 (q)	17.4 (q)	3	78.5 (d)	78.6 (d)
				4	70.7 (d)	71.6 (d)
				5	67.2 (t)	78.4 (d)
				6		62.5 (t)

[M-H-146-162]$^-$, [M-H-146-162-162]$^-$; ^{1}H NMR (pyridine-d_5, 400 MHz,): δ 3.68 (1H, H-3), 4.41 (1H, H-16), 0.89 (3H, s, H-18), 0.72 (3H, s, H-19), 1.20 (3H, d, J=7.0 Hz, H-21), 3.56 (br d, 10.6 Hz, H-26), 3.43 (t, 10.5 Hz, H-26), 0.76 (3H, d, J=5.1 Hz, H-27); 1.70 (3H, d, J=6.3 Hz, H-Rha-6), 4.95 (d, J=7.6 Hz, H-Gal-1), 5.21 (d, J=7.9 Hz, H-Glc-1), 5.27 (d, J=7.6 Hz, H-Glc″-1), 5.57 (d, J=7.9 Hz, H-Glc′-1) and 6.22 (1H, br s, H-Rha-1); ^{13}C NMR data see table 1.

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